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Antibacterial activity of *Securidaca longipedunculata* stem bark against some clinical bacterial isolates

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Abstract
Securidaca longipedunculata is a multipurpose plant with a long history of use in Nigerian and African traditional medicine. The present study was aimed to investigate the antibacterial activity of the aqueous stem bark extract of this important plant against clinical isolates of *Escherichia coli, Salmonella typhi, Staphylococcus aureus, Shigella dysenteriae, Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. The result shows that the extract was active against all the tested bacterial isolates; the highest activity was recorded against *Staphylococcus aureus* (36 mm) and lowest activity against *Shigella dysenteriae* (24 mm). The minimum inhibitory concentration was observed within the range of 2.5-5 mg/ml, while the minimum bactericidal concentration was observed within the range of 5-10 mg/ml. The phytochemical screening revealed the presence of anthraquinones, saponins, phenolic compounds, carbohydrates, glycosides, steroids, terpenoids, alkaloids and flavonoids. The study has supported the use of this plant in the management of bacterial diseases and could serve as a potential source of antibacterial drugs of plant origin.

Keywords: *Securidaca longipedunculata*, clinical isolates, traditional medicine, antibacterial drugs

Introduction
During the last few decades, medicinal plants have attracted the attention of pharmaceutical and scientific communities, and evidence has demonstrated the promising potential of antimicrobial plant derived substances [1], among the new antibacterial drugs approved from 1981-2006; 69 % were originated from natural products, while 21 % of antifungal drugs were natural derivatives or compounds mimicking natural products [2].
The antimicrobial effect of plant oils and extracts has formed the basis of many applications, including raw and processed food preservation, pharmaceuticals and alternative medicine. Plant materials are of wide use in traditional system of medicine, and in several communities of the developing world, are the only resources available for the treatment of different infections. In some Asian and African countries, 80 % of the population depends on traditional medicine for primary healthcare and many have regulations for herbal medicines [3]. Plants are potential sources of new drugs for man whose use to control diseases is a centuries old practice. Currently, numerous reviews have described the importance of natural compounds to treat human diseases [4].
Also, the development of resistance to current antibiotics by disease causing microorganisms has reinforced research discovery of new ones. There is an urgent need for new antimicrobial agents of plant origin to overcome the problem of resistance, in fact, the theme of the World Health Day 2011 was "antimicrobial resistance: no action today, no cure tomorrow" [5].
Securidaca longipedunculata is locally known as Sanya/Uwar Magunguna (Hausa) and Ipeta (Yoruba). It is a shrub that can grow up to 6 m high with a pale grey, smooth bark and oblong alternate leaves that are variable in size and shape and crowded towards the stem tips [6]. It is traditionally used to treat fever, diarrhea, dysentery, typhoid constipation, headaches, rheumatism, malaria, tuberculosis, pain, epilepsy, pneumonia, skin infections urethral discharges, stomach problems, toothache, sleeping sickness, cough, chest complaints, snakebite and wound dressing [7]. The objective this work was to evaluate the antibacterial activity of *S. longipedunculata* stem bark extracts against some clinical bacterial isolates.

Materials and Methods
Microbial Cultures
Cultures of clinical isolates of *Escherichia coli, Salmonella typhi, Staphylococcus aureus, Shigella dysenteriae, Pseudomonas aeruginosa* and *Klebsiella pneumoniae* were obtained from the Department of Medical Microbiology, Aminu Kano Teaching Hospital, Kano State, Nigeria

Identification of the Test Organisms
The clinical isolates were reconfirmed using routine bacteriological and biochemical tests including indole, motility, citrate utilization, urease production, hydrogen sulfide production as well as acid and gas production [8, 9].

Collection, Identification and Preparation of Plant Material
The plant was collected from Gwaram Local Government Area, Jigawa State, Nigeria. It was identified in the field using taxonomic characters and then taken to the Herbarium of Ethnobotany Unit of Bioresource Development Centre, Kano for authentication. A reference voucher number, BDCKN/EB/1898 was deposited in the Herbarium.
The powdered stem bark (100 g) was macerated with distilled water (500 ml) for 48 hours, the mixture was shaken occasionally. The filtrate obtained was evaporated to dryness at 40 ^{0}C using rotary evaporator and water bath.

Preliminary Phytochemical Screening
The preliminary phytochemical screening of the aqueous extract of *S. longipedunculata* stem was conducted using standard qualitative methods [10-13].

Preparation of the Extract for Antibacterial Study
The extract (0.2 g) was dissolved in 10 ml distilled water to obtain a concentration of 20 mg/ml.

Zones of Inhibition
Well diffusion method described by [14] was used to determine the antimicrobial activity (zones of inhibition) of the extract. Mueller-Hinton agar was used to grow the bacterial isolates, the sterilized medium was seeded with 0.1 ml of the standardized inoculum of the test microbe; the inoculum was spread evenly over the surface of the medium using a sterile swab.
A standard cork borer of 6 mm in diameter was used to cut a well at the centre of each inoculated plate, 0.1 ml of the extract (20 mg/ml) was then introduced into the well created at the centre of each plate. The plates were then incubated at 37^{o}C for 24 hours after which each plate was observed for the zone of inhibition of growth. The zone sizes were measured and recorded to the nearest millimeter.

Minimum Inhibitory Concentration (MIC)
The minimum inhibitory concentration (MIC) of the aqueous extract was determined using the broth dilution method as described by [15]. Mueller-Hinton broth was prepared according to manufacturer's instruction (Oxoid Ltd., Basinstoke, Hampshire, England), 10 ml of Mueller-Hinton broth was dispensed into each test tube, sterilized at 121 ^{o}C for 15 minutes and allowed to cool. The inoculum was prepared by suspending an overnight culture of the test microbe in a normal saline until the turbidity matches that of McFarland turbidity standard (McFarland 0.5) which contains approximately1.5x10^{8}cfu/ml. Two fold serial dilution of the extract in the sterile broth was made to obtain the concentrations of 20 mg/ml, 10 mg/ml, 5 mg/ml, 2.5 mg/ml and 1.25 mg/ml, 0.1 ml of the test inoculum was then transferred from the normal saline to the different concentrations. The test tubes were then incubated at 37 ^{o}C for 24 hours after which they were observed for growth (turbidity). The lowest concentration of the extract in the broth which shows no turbidity was recorded as the minimum inhibitory concentration [15].

Minimum Bactericidal Concentration (MBC)
The content of the MIC tubes in the serial dilution were sub-cultured on to the prepared medium and incubated at 37^{o}C for 24 hours, after which the plates were observed for the presence of colonies, the minimum bactericidal concentrations were the plates with lowest concentration of the extract without bacterial colony [15].

Results
Preliminary Phytochemical Screening
Anthraquinones, saponins, phenolic compounds, carbohydrates, glycosides, steroids, terpenoids, alkaloids and flavonoids were all detected in the aqueous extract of *S. longipedunculata* stem bark (Table 1).

Table 1: Phytochemical Constituents

Phytochemicals	Inference
Tannins	Absent
Anthraquinones	Present
Glycosides	Present
Saponins	Present
Phenolic compounds	Present
Flavonoids	Present
Alkaloids	Present
Terpenoids	Present
Steroids	Present
Carbohydrates	Present

Zones of Inhibition
The aqueous extract of *S. longipedunculata* stem bark was found to be active against all the test organisms, with the highest activity against *S. aureus* (36 mm) and lowest activity against *Shigella dysenteriae* (24 mm) (Table 2).

Table 2: Different Zones of Inhibition of the Extract against the Test Microbes (mm)

Test Organism	Extract (20 mg/ml)	Standard Drug Ciprofloxacin(5µg)
Staphylococcus aureus	36	48
Escherichia coli	32	42
Salmonella typhi	28	45
Pseudonomonas aeruginosa	28	35
Shigella dysenteriae	24	50
Klebsiella pneumoniae	30	43

Minimum Inhibitory Concentration
The minimum inhibitory concentration (MIC) of the aqueous extract of *S. longipedunculata* stem bark was observed within the range of 2.5-5 mg/ml as summarized Table 3:

Table 3: Minimum Inhibitory Concentration of the Extract against the Test Microbes

Test Organism	20 mg/ml	10 mg/ml	5 mg/ml	2.5 mg/ml	1.25 mg/ml
Staphylococcus aureus	-	-	-	*	+
Escherichia coli	-	-	-	*	+
Salmonella typhi	-	-	-	*	+
Pseudomonas aeruginosa	-	-	-	*	+
Shigella dysenteriae	-	-	*	+	+
Klebsiella pneumoniae	-	-	-	*	+

Key:
*= MIC
- = No growth
+ = Growth

Minimum Bactericidal Concentration
The minimum bactericidal concentration (MBC) of the

aqueous extract of *S. longipedunculata* stem bark of was observed within the range of 5-10 mg/ml (Table 4).

Table 4: Minimum Bactericidal Concentration of the Extract against the Test Microbes

Test Organism	20 mg/ml	10 mg/ml	5 mg/ml	2.5 mg/ml	1.25 mg/ml
Staphylococcus aureus	-	-	*	+	+
Escherichia coli	-	-	*	+	+
Salmonella typhi	-	-	*	+	+
Pseudomonas aeruginosa	-	-	*	+	+
Shigella dysenteriae	-	*	+	+	+
Klebsiella pneumoniae	-	-	*	+	+

Key:
*= MBC
-= No growth
+= Growth

Discussion

The phytoconstituents detected in the stem bark of *S. longipedunculata* were also reported to be present in different extracts of leaf and root of the plant. Tannins were also found to be absent in the chloroform, methanol and aqueous root extracts of the violet tree [16, 17].

The efficacy of the extract against the test isolates could be attributed to the presence of some bioactive compounds [18]. Several studies have shown that flavonoids, saponins, alkaloids and tannins were reported to be responsible for the antibacterial and/or antimicrobial activity associated with many medicinal plants, thus, the antibacterial property observed in this study could be attributed to the flavonoids, alkaloids and saponins detected [19-21].

The antibacterial study has shown that all the test isolates were sensitive to the aqueous stem bark extract of *S. longipedunculata* as zones of inhibition were produced at the tested concentration. The extract exhibits highest activity against *S. aureus* and lowest activity against *dysenteriae*. The recorded zones of inhibition for *S. aureus*, *E. coli*, *P. aeruginosa* and *Salmonella typhi* were higher than those reported for leaf and root bark extracts of *S. longipedunculata*. Previous studies also revealed that *Salmonella typhi* and *P. aeruginosa* were resistant to the chloroform, methanol and aqueous root extract of the plant, and this suggests that the stem bark contains more antibacterial agents than the other parts of *S. longipedunculata* [16, 17, 22].

Table 3 and 4 showed the minimum inhibitory and minimum bactericidal concentrations of the aqueous extract of *S. longipedunculata* stem bark. The results indicates that the extract is more effective against *E. coli*, *S. typhi*, *S. aureus*, *P. aeruginosa* and *K. pneumonia* (MIC, 2.5 mg/ml and MBC, 5 mg/ml) than *S. dysenteriae* (MIC, 5 mg/ml and MBC, 10 mg/ml). However, the minimum inhibitory concentrations observed in this study were higher than those reported by [16], but lower than those reported by [17].

Conclusion

The study has shown that the stem bark extract of *S. longipedunculata* possess strong antibacterial property, and this may account for its use in traditional medicine in the management of bacterial diseases, therefore, *S. longipedunculata* could serve as a potential source of antibacterial agents of plants origin.

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